

### DETAILED ACTION

Applicants submit this amendment cancelling 66-84 without prejudice or disclaimer.

### EXAMINER'S COMMENTS

The preliminary amendment filed on 06/02/03 is acknowledged. Claims 57-68 are now pending in this application

### APPLICANTS' STATEMENT

Applicants amend independent claims 57 and 61 to specify an apparatus that serves to set up protein crystallization experiments in multiwell plates with sitting drop and hanging drop formats respectively. The independent claims also specify that protein crystallization experiments are formed by the apparatus having a total volume less than 1  $\mu$ L capable of forming protein crystals suitable for X-ray diffraction.

Prior to Applicants' invention, the world conducted protein crystallization experiments in volumes greater than 1  $\mu$ L. This was in part due to the expectation that protein crystallization experiments require greater than 1  $\mu$ L. As evidence of this perception, Applicants enclose Berry, M., Protein Crystallization: Theory and Practice (1995). The subsection of Berry entitled "Physical methods of crystallization" teaches at paragraph 5 that:

"One of the drawbacks to vapor equilibrium is that it tends to form smaller crystals than other methods. This may be due to small drop volumes limiting the quantity of crystallizable solute present or creating a higher level of impurities relative to other techniques which utilize larger volumes. As discussed earlier in section 2.2., as crystals grow, the concentration of defective molecules increases relative to perfect molecules (which are being selected for by the crystal). When this factor is combined with the higher probability of impurities diffusing to the face of the crystal (due to the smaller volumes), the likelihood of inclusion of defects into the growing crystal increases. Thus, the production of X-ray quality crystals may be better suited to the use of batch, free interface diffusion, or dialysis techniques which utilize larger solution volumes at equilibrium."

As can be clearly seen from the above passage, Berry teaches that the relative concentration of impurities in a protein crystallization experiment increases as the size of the experiment is reduced. These impurities function to impede protein crystal growth and deleteriously affect the

X-ray diffracting ability of the resulting crystal. As a result, Berry teaches that “batch, free interface diffusion, or dialysis techniques which utilize larger solution volumes at equilibrium” are more desirable than vapor diffusion techniques such as sitting and hanging drop which utilize small volumes.

The Examiner’s attention is also drawn to Mueller, et al., Journal of Biotechnology 85 (2001) 7-14, also enclosed. At page 13, left column, Mueller, et al. teaches that “the experiments shown here [250nL in size] illustrate clearly that a miniaturized set-up is possible” and that “interestingly, the crystals obtained in 250 nL droplets show the same morphology as in other experiments but they were always smaller in size.” The fact that Mueller, et al. felt that it was worth while to publish the unexpected result that experiments in 250nL are “possible” and that “interestingly”, the same crystals are formed, independently confirms the unexpected nature of the presently claimed invention.

Applicants submit that the relevant protein crystallization art has other examples of references teaching away from performing protein crystallization experiments in volumes less than 1  $\mu$ L and are currently in the process of compiling further such examples.

Given the expectation in the art prior to Applicants’ priority date that a volume greater than 1  $\mu$ L was needed, the general desire to reduce experiment sizes cited by the Examiner is not sufficient in and of itself to negate the inventiveness of Applicants’ use of submicroliter volumes. The Examiner is therefore asked to recognize both the novelty and inventiveness of the “less than 1 microliter” language that is required in all of the claims. In view of this material distinction, Applicants further request that the Examiner withdraw all of the pending rejections for obviousness since none of the references cited of record teach or suggest this facet of the claimed invention.

#### INFORMATION DISCLOSURE STATEMENTS

The Examiner states that:

“Due to overwhelming volume of IDS submitted for the instant application the

examiner requests indicating relevance of the prior art to the subject matter of the present application and withdrawing irrelevant references from IDS in future submissions of IDS.”

Applicants have submitted a large number of references for this patent application and in the related pending patent applications. In this important technology, these references were submitted to assure that all pertinent references were reviewed and cited by the Examiner.

### REJECTIONS UNDER 35 USC 112

The Examiner states that:

“The specification is objected to as not being written “in such full, clear, concise, and exact terms as to enable any person skilled in the art to” practice the invention in its best mode.

Throughout the specification the term “mother liquor” is used in the context, which appears to contradict conventional meaning of this term. According to on-line Dictionary.com: “*mother liquor* is the impure or complex residual solution which remains after the salts readily or regularly crystallizing have been removed”; on-line MSDS HyperGlossary: “When a solid deposits from a solution the process is called crystallization if the solid is crystalline and precipitation if the solid is a powder or amorphous material. The solid that is formed is called the precipitate and the liquid solution that remains is called *the supernatant or “mother liquor”*”. It is not clear from the specification, how operations involving “mother liquid” are related to the subject matter of the invention, i.e. the method and apparatus for crystallizing samples. As it is mentioned, the “mother liquor” is the solution remained after crystallization when the crystals are removed, and therefore it is not apparent, how it can be used in the crystallization experiments. If the Applicants are using this term in a non-conventional manner, its definition should be clearly indicated in the specification.

The specification contains a plurality of typos, particularly related to the volume of the samples: it seems that in all cases the Greek letter  $\mu$  is absent from  $\mu\text{L}$ , thus leaving L as a volume measure (starting with page 4). Appropriate corrections are required.”

Applicants traverse the Examiner’s objection to the use of “mother liquor” in the Specification and the claims.

Applicants note that the term “mother liquor” is used consistently throughout the Specification and consistently with regard to its usage in the protein crystallization arts. As discussed during Applicants’ interview with the Examiner on June 1, 2005, “mother liquor” is a term known in the protein crystallization arts as evidenced by its usage in references cited by Applicants via already filed Information Disclosure Statements, as well as by conducting a simple Google search for [“protein crystallization” and “mother liquor”]. The Examiner is therefore respectfully requested to withdraw this ground of rejection.

Applicants have amended the uL terms with the Preliminary Amendment filed in most copending applications. An amendment regarding these amendments for typographical errors will be in this file shortly..

For these reasons Applicants argue these rejections have been overcome.

Reconsideration and withdrawal of the rejection are respectfully requested.

#### REJECTIONS OF CLAIMS 72-84 PURSUANT TO 35 USC 112

Claims 73-84 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter, which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The Examiner states that:

“Claims 73, 77 and 81 recite a mother liquor drop station capable of simultaneous removing mother liquor from a plurality of wells and delivering it to drop regions (“at the same time”). The specification does not disclose an apparatus capable of simultaneous removing solutions from the microtiter plate and delivering them to a different area, since these operations can only be sequential. Thus, claims 73-84 are not enabled by the specification.”

Applicants respectfully traverse this rejection.

Claims 73-84 are now cancelled without prejudice or disclaimer.

Therefore this rejection has been obviated.

REJECTIONS OF CLAIMS 57-84 PURSUANT UNDER 35 USC 112 (2nd)

Claims 57-84 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The Examiner states:

“Claims 57-84 recite manipulation with “mother liquor”, which has unclear meaning in the context of the present application. This renders the claims unclear and indefinite, since it is not apparent from the specification, what this liquor is, and how the apparatus is supposed to operate in order to properly manipulate with it. For example, if the “mother liquor” is the solution formed after crystallization, does it mean that “a mother liquor station capable of removing mother liquor from a plurality of wells” should be capable of separating mother liquor, or supernatant, from the crystals?

From the claims it is not clear, what is the difference between two “drop stations”: “a mother liquor drop station” and “a molecule drop station”, which both should be capable of delivering samples to the same place. Moreover, since claims 70, 72, 73, and 81 recite in their bodies only stations capable of withdrawing solutions of volumes less than 25 nL from the wells of microtiter plate and delivering them to a different location, the examiner considers all fluid handling apparatus capable of these operations meeting the requirements of the pending claims.”

Applicants respectfully traverse this rejection.

Applicants’ statements above and arguments above for the rejections to the specification are incorporated by reference at this point.

The terms “drop stations”, “a mother liquor drop station” and a “molecule drop station” are defined in the application and understood by one of skill in the art.

Applicants request this rejection be withdrawn.

REJECTIONS OF CLAIMS 57-72 PURSUANT TO 35 USC § 103

Claims 57-60, 66-70 and 72 are rejected under 35 U.S.C. 103(a) as being unpatentable over any of Hayes et al. (US 5,658,802), Ershow et al. (US 5,756,050), Brown et al (US

5,807,522), Tisone et al. (US 6,063,339), Papen et al. (US 6,079,283), Little et al. (US 2001/0008615A1) or Bienert et al. (US 2001/0019845A1).

(These references are cited below as being SUBPARAGRAPH 12.)

The Examiner states that:

“All indicated references disclose fluid handling systems capable of delivering plurality of samples withdrawn from multiwell plates in volumes less than 25 nL (20 nL, 15 nL), the system comprising a platform on which a multiwell plate is positionable. The systems are capable of delivering nanoliter drops into microtiter plate wells, on substrates, etc., which would include sitting drop regions. Piezoelectric valve or solenoid valve is conventionally used in these systems.

Although the references do not specifically indicate two fluid handling systems with similar capabilities, it would have been obvious for anyone of ordinary skill in the art to use two such systems, because this expands the area of application of such devices, including delivering nanoliter volumes of reagents from different reagent sources.”

Applicants respectfully traverse this rejection.

This rejection is interpreted to be a 35 USC 103 rejection for each of these references and not for any combination thereof.

Applicants’ statement above and prior arguments are incorporated by reference at this point.

Hayes et al. disclose a method and apparatus for making miniaturized diagnostic arrays. An examination of the specification and the figures indicate that it does not teach or suggest the present claims.

Ershow et al. disclose a device for dispensing microdoses of aqueous solutions of substances onto a carrier and a device for carrying out the method.

Brown et al. disclose a liquid chemical distribution method and apparatus.

Tisone et al. disclose a method and apparatus for high speed dot array dispensing.

Papin et al. disclose a method for aspirating a sample liquid into a dispenser tip and thereafter ejecting droplets.

Little et al. disclose systems and methods for preparing and analyzing low volume analyte array elements.

Bienert et al. disclose a metering head for parallel processing of a plurality of fluid samples.

Again after an examination of the specification and figures of these cited references it is apparent that individually the present invention is not taught or suggested.

Therefore this rejection has been overcome.

Reconsideration and withdrawal are respectfully requested.

REJECTIONS OF CLAIMS 61-65 AND 71 UNDER TO 35 USC § 103

Claims 61-65 and 71 are rejected under 35 U.S.C. 103(a) as being unpatentable over any of the prior art applied to claims 57-60, 66-70 and 72 above, in view of Meltzer (US 5,873,394, IDS).

The Examiner states that:

“While none of the prior art recited above discloses a cover slip station, Meltzer discloses “automated sample preparation workstation for the vapor diffusion method of crystallization and method of preparation” (Title), with the apparatus comprising a cover slip station and fluid devices capable of delivering 1-10  $\mu$ L volumes of the compound to be crystallized (“a molecule drop”) and the mixing compound (“mother liquor”) to the plurality of cover slips.

It would have been obvious for any person of ordinary skill in the art to apply fluid handling systems of the prior art recited above in Meltzer’s automated sample preparation and crystallization because this application is one of numerous applications requiring handling of fluids in extremely small volumes, as indicated by Meltzer.”

Applicants respectfully traverse this rejection.

This rejection is interpreted to be a combination of any of the individual cited references in combination with Meltzer.

Applicants’ statements and arguments immediately above are incorporated herein by reference for each cited reference.

Meltzer discloses an automated sample preparation workstation for the vapor diffusion method of crystallization and method of preparation. There is no teaching of successful submicroliter quantity transfer for microcrystallization.

Applicants can find no teaching or suggestion to combine the prior cited references with the teachings of Meltzer to obtain Applicants’ present invention.

This rejection is overcome.

Reconsideration and withdrawal are respectfully requested.

REJECTIONS OF CLAIMS 57-65 AND 70-72 UNDER 35 USC § 103

Claims 57-65 and 70-72 are rejected under 35 U.S.C. 103(a) as being unpatentable over Meltzer in view of any of the prior art cited in subparagraph 12.

The Examiner states that:

“Meltzer discloses “an automated sample preparation workstation and sample preparation method for the vapor diffusion method of crystallization which performs multiple processing functions such as pipetting, diluting, coverslip manipulations, plate greasing and the like under microprocessor control. The apparatus is responsive to operator-entered processing requests and performs complex and accurate processing functions. The disclosed apparatus is menu-driven and thus easy to learn and simple to operate” (Title). In particular, Meltzer discloses “9. The small pipetting probe aspirates a small amount (1 to 10 ul’s) of the compound to be crystallized from a vial and dispenses it onto the coverslip 10. The small pipetting probe aspirates a small amount (1 to 10 ul’s) from the well containing the mixing compound and places it onto the coverslip with the compound to be crystallized and mixes it” (col. 4, lines 38-44). While Meltzer does not disclose fluid handling of smaller amounts of the samples, less than 25 nL, the prior art cited in subparagraph 12 discloses just that.

It would have been obvious for any of ordinary skill in the art to use fluid handling devices capable of manipulating with liquids of volumes less than 25 nL, as disclosed in the prior art of subparagraph 10 in Meltzer’s crystallization workstation, because this allows delivery of more precise and accurate volumes, decreases time for crystallization, and increases the number of experimental sites due to a smaller volume of each site.”

Applicants respectfully traverse this rejection.

This rejection is interpreted to have Meltzer as the primary reference with the prior cited reference of SUBPARAGRAPH 12 each as secondary references.

Applicants’ statement and arguments above for Meltzer are incorporated by reference.

Applicants’ arguments above for each of the references of SUBPARAGRAPH 12 are incorporated herein by reference.

Again Applicants argue that Meltzer in combination with each of these cited references does not have any specific language to combine the references to teach or suggest Applicants’ present invention.



Therefore this rejection has been overcome.

Reconsideration and withdrawal are respectfully requested.

REJECTIONS OF CLAIMS 66-69 UNDER TO 35 USC § 103(a)

Claims 66-69 are rejected under 35 U.S.C. 103(a) as being unpatentable over McPherson et al. (US 5,096,676, IDS) in view of any of the prior art cited in subparagraph 12.

The Examiner states that:

“McPherson et al. discloses ‘an apparatus for growing diffraction quality, protein crystals by vapor diffusion techniques’, involving ‘sitting drop’ method (Abstract). “The apparatus of the present invention overcomes many of the drawbacks of the prior art methods and devices by providing novel crystal growing apparatus which is inexpensive, easy to use and readily compatible with automatic pipetting, sample preparation and diffraction analysis equipment. Because of the unique design of the apparatus, laboratory technicians with no particular skills, or specialized training, can use the apparatus to grow high quality crystals in very large numbers for numerous uses, including the diffraction analysis of macromolecular structures.

McPherson does not specifically disclose fluid handling apparatus capable of operating very small volumes of solutions (less than 15 nL). The prior art cited in subparagraph 12 discloses exactly such systems. It would have been obvious for any person of ordinary skill in the art to use modern sophisticated apparatus of the prior art recited in subparagraph 12 with McPherson’s crystal growing apparatus, because McPherson specifically indicates that his apparatus is “easy to use and readily compatible with automatic pipetting, sample preparation and diffraction analysis equipment”, and because the fluid delivery apparatus of the cited prior art are capable of delivering extremely precise volumes of solutions into the sitting drop microwells of McPherson’s apparatus, which is very important for obtaining uniform crystals for X-ray analysis.”

Applicants respectfully traverse this rejection.

This rejection is interpreted to have McPherson et al. as the primary reference in combination with each of the references cited above in SUBPARAGRAPH 12.

McPherson et al. discloses a crystal growing apparatus. A careful reading of the specification, claims and figures does not teach or suggest the submicroliter levels of Applicants’ present invention.

Applicants’ statement and the arguments above for each references cited in SUBPARAGRAPH 12 is incorporated by references at this point.

Again a careful reading of each of these cited references does not specifically reference any other reference and does not teach or suggest how the combination would result in Applicants' present invention at submicrolevels.

Therefore this rejection has been overcome.

Reconsideration and withdrawal are respectfully requested.

#### FURTHER REMARKS

Applicants submit this amendment cancelling claims 66-84 without prejudice or disclaimer. Applicants note that cancelled claims 73 to 84 are now found in pending U.S. Ser. No. 10/323,054.

#### SUMMARY

Applicants argue the pending claims here are of a form and scope for allowance. Prompt notification thereof is respectfully requested.

If there are any questions, the Examiner is requested as soon as possible to call the undersigned attorney of record Howard Peters, (650) 324-1677 ext. 103.

If additional fees are required for the filing of these documents, the Commissioner of hereby authorized to charge or credit an overpayment to Deposit Account No. 16-1331.

Respectfully submitted,

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